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Use of the rabbit ear artery to serially assess foreign protein secretion after site-specific arterial gene transfer in vivo. Evidence that anatomic identification of successful gene transfer may underestimate the potential magnitude of transgene expression.

Losordo DW, Pickering JG, Takeshita S, Leclerc G, Gal D, Weir L, Kearney M, Jekanowski J, Isner JM.

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BACKGROUND: The development of molecular strategies for the treatment of restenosis has been hindered by low efficiencies of in vivo arterial transfection. Expression of intracellular marker proteins is generally evident in < 1% of vascular smooth muscle cells after in vivo arterial transfection. Efforts to improve the efficiency of in vivo gene transfer have been further impeded by the use of transgenes encoding for intracellular marker proteins, necessitating tissue removal and limiting survey for expression to one point in time. **METHODS AND RESULTS:** To study gene expression on a serial basis in vivo and determine the relation between a secreted gene product and transfection efficiency after in vivo arterial gene transfer, a method for performing and serially monitoring gene expression in vivo was developed using the central artery of the rabbit ear. Liposome-mediated transfection of plasmid DNA containing the gene for human growth hormone (hGH) was successfully performed in 18 of 23 arteries. Serum hGH levels measured 5 days after transfection ranged from 0.1 to 3.8 ng/mL (mean, 0.97 ng/mL); in contrast, serum drawn from the control arteries demonstrated no evidence of hGH production. Serial measurement of hGH from transfected arteries demonstrated maximum hGH secretion 5 days after transfection and no detectable hormone after 20 days. Despite these levels of secreted gene product documented in vivo, immunohistochemical staining of sections taken from the rabbit ear artery at necropsy disclosed only rare cells in which there was evidence of successful transfection. **CONCLUSIONS:** These experiments demonstrate a useful method of performing serial in vivo analyses of gene expression after vascular transfection and that anatomic analyses of

transfection efficiency may underestimate the potential magnitude of expression in the case of a secreted gene product. These findings have implications for the clinical application of somatic gene therapy because low-efficiency transfection with a gene encoding for a secreted protein may achieve therapeutic effects not realized by transfection with genes encoding for proteins that remain intracellular.

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